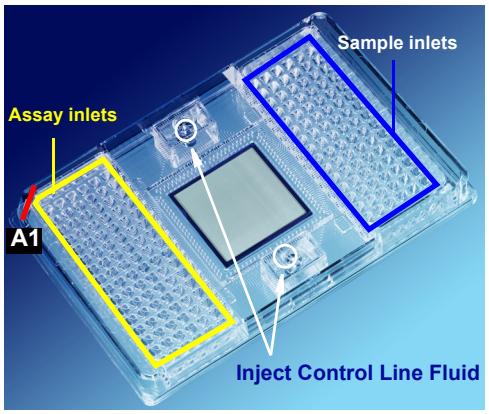
Fluidigm® 96.96 Real-Time PCR Workflow Quick Reference

PN 68000130, Rev. B

For more information, see the *BioMark Real-Time PCR Analysis Software User Guide, PN 68000088*

1 Priming the 96.96 Dynamic Array™ IFC



CAUTION! USE THE 96.96 CHIP WITHIN 24 HOURS OF OPENING THE PACKAGE.

- DUE TO DIFFERENT ACCUMULATOR VOLUMES, ONLY USE 96.96 SYRINGES WITH 150 µL OF CONTROL LINE FLUID.
- CONTROL LINE FLUID ON THE CHIP OR IN THE INLETS MAKES THE CHIP UNUSABLE.
- LOAD THE CHIP WITHIN 60 MINUTES OF PRIMING.

1 Inject control line fluid into each accumulator on the chip.

2 Place the chip into the IFC (integrated fluidic circuit) controller, then run the ***Prime (136x)*** script to prime the control line fluid into the chip.

2 Preparing 10x Assays

In a DNA-free hood, prepare 5 µL aliquots of 10X assays using the volumes in the table below (scale up appropriately for multiple runs).

Component	Volume per Inlet (µL)
20x TaqMan® Gene Expression Assay (Applied Biosystems)	2.5
2x Assay Loading Reagent (Fluidigm, PN 85000736)	2.5
Total Volume	**5.0**
Final Concentration (at 10x)	**Primers: 9 µM; Probe: 2 µM**

3 Preparing Sample Pre-Mix and Samples

1 Combine the components in the table below to make the Sample Pre-Mix and the final Sample Mixture (scale up appropriately for multiple runs).

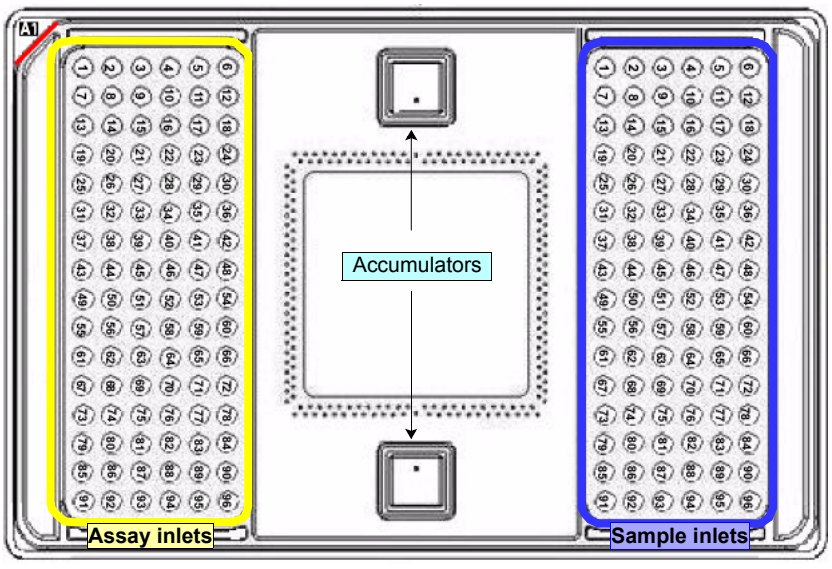
SAMPLE PRE-MIX

Component	Volume per Inlet (µL)
TaqMan® Universal PCR Master Mix (2x) (Applied Biosystems, PN 4304437)	2.5
20x GE Sample Loading Reagent (Fluidigm, PN 85000746)	0.25
cDNA	2.25
Total Volume	**5.0**

2 In a DNA-free hood, combine the TaqMan Universal PCR Master Mix with the GE Sample Loading Reagent in a 1.5 mL sterile tube—enough volume to fill an entire chip. 2.75 µL of this Pre-Sample Mix can then be aliquoted for each sample.

3 Remove these aliquots from the DNA-free hood and add 2.25 µL of cDNA to each, making a total volume of 5 µL in each aliquot.

Chip Pipetting Map



Technical Support

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4 Loading the Chip

IMPORTANT! MAKE SURE YOU THOROUGHLY MIX ALL ASSAY SOLUTIONS AND ALL SAMPLES *BEFORE* PIPETTING INTO THE CHIP INLETS.

1 When the *Prime (136x)* script has finished, remove the primed chip from the IFC controller and pipette 5 µL of each assay and each sample into their respective inlets on the chip.

IMPORTANT! FOR UNUSED SAMPLE INLETS, USE 2.75 µL OF SAMPLE MIX AND 2.25 µL OF DNA-FREE WATER PER INLET. FOR UNUSED ASSAY INLETS, USE 2.5 µL ASSAY LOADING REAGENT AND 2.5 µL WATER

CAUTION! WHILE PIPETTING, *DO NOT* GO PAST THE FIRST STOP ON THE PIPETTE. DOING SO MAY INTRODUCE AIR BUBBLES INTO INLETS.

2 Return the chip to the IFC controller.

3 Using the IFC controller software, run the **Load Mix (136x)** script to load the samples and assays into the chip.

4 When the *Load Mix (136x)* script has finished, remove loaded chip from the IFC controller.

5 Peel the blue protective film from the underside of the loaded chip.

6 Remove any dust particles or debris from the chip surface.

You are now ready for your chip run.

CAUTION! START THE CHIP RUN ON THE INSTRUMENT IMMEDIATELY AFTER LOADING THE SAMPLES.

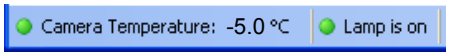
5 Using the Data Collection Software

IMPORTANT! BE SURE TO SELECT *ALL* PROBE TYPES PRESENT IN YOUR EXPERIMENT. DATA ARE NOT COLLECTED ON UNSPECIFIED PROBES.

1 Double-click the Data Collection Software icon on the desktop to launch the software.

2 Click **Start a New Run**.

3 Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding.



4 Place the chip into the reader.

5 Click **Load**.

6 Verify chip barcode and chip type.

a Choose project settings (if applicable).

b Click **Next**.

7 Chip Run file:

a Select **New** or **Predefined**.

b Browse to a file location for data storage.

c Click **Next**.

8 Application, Reference, Probes:

a Select Application Type—**Gene Expression**.

b Select Passive Reference **(ROX)**.

c Select Assay—**Single probe**, **Two probes** or **More than two probes**.

d Select probe types.

e Click **Next**.

9 Click **Browse** to find thermal protocol file—**M96 default protocol.pcl**.

CAUTION! MAKE SURE THAT YOU USE A 96.96 SPECIFIC PROTOCOL.

10 Confirm **Auto Exposure** is selected.

11 Click **Next**.

12 Verify the chip run information.

13 Click **Start Run**.

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